VIVEKANANDA COLLEGE
THAKURPUKUR
KOLKATA-700063

NAAC ACCREDITED 'A' GRADE

Topic:Techniques for isolation and analysis of protein

Course Title: Proteins and enzymes

Paper:GE-2 (CC-2)

Unit:I

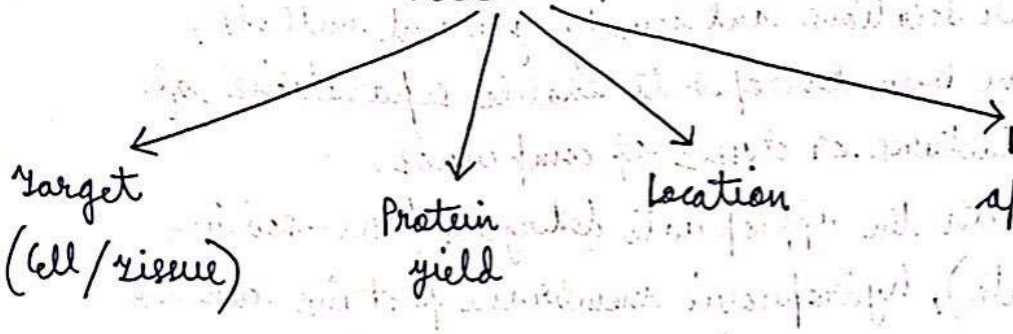
Semester:2

Name of the Teacher:Nineesha Sen Banerjee

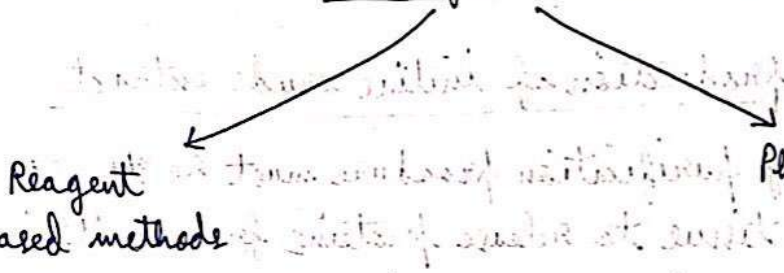
Name of the Department:Biochemistry

6/2/19

Cell Isolation



Cell lysis method



All cells have a plasma membrane, a protein-lipid bilayer that forms a barrier separating cell contents from the extracellular environment. Lipids comprising the plasma membrane are amphipathic, having hydrophilic and hydrophobic moieties that associate spontaneously to form a closed bimembrane sheet. Membrane proteins are embedded in the lipid bilayer, held in place by one or more domains spanning the hydrophobic core.

In addition, peripheral proteins bind the inner or outer surface of the bilayer through interactions with integral membrane proteins or with polar lipid head groups.

In animal cells, the plasma membrane is the only barrier separating cell contents from the environment, but in plants and bacteria, the plasma membrane is also surrounded by a rigid cell wall.

Examples: Bacterial cell walls are composed of peptidoglycan. Yeast cell walls are composed of 2 layers of β-glucan and plant cell walls consist of multiple layers of cellulose.

Quality or physical form of the isolated protein is also an important consideration when extracting proteins for certain downstream applications. Example: Crystallography.

By careful optimisation of physical disruption techniques, detergent-buffer solutions and density gradient methods, procedures have been developed to enable separations of subcellular structures or classes of compounds.

For example: With the appropriate detergents (SDS - sodium dodecyl sulphate), hydrophobic membrane proteins can be solubilized and separated from hydrophilic proteins.

Cell disruption and production of initial crude extract

The initial step of any purification procedure must be ~~to~~ to disrupt the starting tissue to release proteins from within the cell. The means of disrupting the tissue will depend on the cell type, but the composition of the buffer used to extract the proteins must be considered. Normally, extraction buffers are at an ionic strength (0·1 - 0·2 molar (M)) and pH 7-8, that is considered to be compatible with that found inside the cell. Tris or phosphate buffers are most commonly used. In addition, an antioxidant, example β-mercaptoethanol, enzyme inhibitors - protease inhibitor.

Example: Diisopropylfluorophosphate (DFP), example: lysosome (cell organelle); EDTA (chelating agent - ethylene diamine tetra acetate); sodium azide (bacteriostatic agent).

Several methods are commonly used including mechanical disruption, liqui frequency sound waves (sonication), fre grinding.

Techniques of protein extractio

Lysis method	Apparatus
1) Mechanical	Waring blender Polytron
2) Liquid homogenization	Dounce ~~homogen~~ homogenizer, Potter-Elvehjem homogenizer, French Press
3) Sonication	-Sonicator
4) Freeze-thaw	Freezer or dry ice with ethanol

1) Mechanical disruption

Mechanical methods rely on the use of rotating blades to grind and disperse large amounts of complex tissues such as liver or muscle. The waring blender and the polytron are commonly used for this purpose. Unlike the waring blender which is similar to standard household blender, the polytron draws tissue into a long shaft containing rotating blades, which can be used with samples as small as 1 ml.

2) Homogenisation

Homogenisation is the process of converting two immiscible liquids into an emulsion. 2 types of homogenisations are distinguished:

i) Primary homogenisation — When the emulsion is created directly from separate liquids.

ii) Secondary homogenisation — When the emulsion is created by reduction in size of droplets in an existing emulsion.

Liquid homogenisation

~~Three~~ different cells are lysed by forcing the cells or tissues suspension through a narrow space, thereby shearing the cell membranes. Three different types of homogenizers are

differential solubilities in different solvents such as water and organic solvents like acetone or hexane.

Addition of water miscible neutral organic solvent particularly ether or acetone decreases the solubility of most globular proteins in water such that they precipitate out.

Protein solubility at a fixed pH and ionic strength is a function of the dielectric constant of the medium. Low dielectric constant lower the solvating power of their aqueous solution of dissolved ions such as proteins.

Water has a relatively high dielectric constant whereas C_6H_6 has a low value. The large class of substrates that are non ionic, but polar compounds, like sugar readily dissolves in water.

Since ethanol has a lower dielectric constant than water; its addition to an aqueous protein solution increases the attractive forces between opposite charges, thus decrease in degree of ionisation of R groups of proteins. As a result, the protein molecules tend to aggregate and precipitate.

Application

Liquid column fractionation used for

1) coal liquefaction and petroleum products used in palm oil manufacture and obtaining confectionary fat from palm oil.
2) It is an useful tool of separation in food and biotechnology industry.
3) In oil industry, it is used to determine the triglyceride composition of palm oil.